Saturable elimination of piperacillin in critically ill patients

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Abstract: Purpose: To evaluate saturation of piperacillin elimination in adult critically ill patients.
Patients and methods: Seventeen adult critically ill patients received continuous and intermittent infusion piperacillin/tazobactam. Piperacillin plasma concentrations (n=217) were analyzed using population pharmacokinetic (PopPK) modeling. Post hoc simulations were performed to evaluate the type I error rate associated with our study. Unseen data was used to validate the final model. The mean error (ME) and root mean squared error (RMSE) were calculated as a measure of bias and imprecision respectively.
Results: A PopPK model with parallel linear and non-linear elimination best fitted our data. The median and 95% confidence intervals for model parameters drug clearance (CL), volume of the central compartment (V), volume of the peripheral compartment (Vp) and intercompartmental clearance (Q) were 9 (7.69 - 11) L/h, 6.18 (4.93 - 11.2) L, 11.17 (7.26 - 12) L and 15.61 (12.66 - 23.8) L/h. The Michaelis-Menten constant (Km) and the maximum elimination rate for Michaelis-Menten elimination (Vmax) were estimated without population variability in the model to avoid overfitting and inflation of the type I error rate. The population estimates for Km and Vmax were 37.09 mg/L and 353.57 mg/h respectively. The ME was -20.8 (95% CI -26.2; -15.4) mg/L while imprecision (RMSE) was 49.2 (95% CI 41.2; 56) mg/L
Conclusion: Piperacillin elimination is (partially) saturable. Moreover, the population estimate for Km lies within the therapeutic window and therefore saturation of elimination should be accounted for when defining optimum dosing regimens for piperacillin in critically ill patients.

Dear Editor,

I am writing to resubmit our revised manuscript entitled, “Saturable elimination of piperacillin in critically ill patients: implications for continuous infusion”, for consideration for publication in the *International Journal of Antimicrobial Agents*.

We have carefully reviewed the suggestions of reviewer #1. All questions have been addressed and changes in the manuscript and figures have been made where necessary.
A clean version as well as a version with the changes highlighted in yellow are submitted.

This manuscript describes original work and is not under consideration by any other journal.
All authors approved the revised manuscript and this submission.

Thank you for receiving our revised manuscript and considering it for review. We appreciate your time and look forward to your response.

Kind regards,

Sofie Dhaese, MD

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Reply to the reviewers' comments

1. As pointed by the author, the study has several short comes: the between-subject variability was not estimated for Km and Vmax; urine samples were not collect impeding the determination of the renal and non-renal clearance; the final popPK model presented a bias towards underpredicting PIP concentrations; a trend in PIP clearance over time could not be excluded due to the experimental design (all patients received continuous infusion first followed by intermittent infusion).

Answer: we have carefully listed the shortcomings of our paper as we believe this information is vital for the interpretation of our results.

We did not collect urine samples to determine the renal and non-renal component of piperacillin clearance but this does not impede the evaluation of whether or not piperacillin clearance is nonlinear. The potential bias because of a trend in PIP clearance over time is indeed inherent to our study design. However, the time interval between the measurements was minimal. Also, to our knowledge, very few PK studies have used a design with random assignment to either intermittent or continuous infusion and a switch after a certain time to evaluate the behavior of piperacillin clearance. Aside from shortcomings, our study also has some specific strengths, not specifically mentioned in our manuscript. We have listed our strengths in response to the general comment of reviewer #1:

a) Type-I error calculations.

   We have used a network with a very large computational power to be able to determine our type-I error rate. The type-I error rate, in our case 6.6%, tells us something about the probability to falsely reject the zero hypothesis (H_0, i.e.

piperacillin clearance is linear). Overfitting of data, which happens when one wants to estimate too many parameters with too little information, may lead to overly optimistic results. In order to obtain a low type-I error rate, we needed to reduce the number of estimated parameters and hence we were unable to estimate the BSV on Km and Vmax. Our low type-I error rate indicates that we have a low probability to falsely conclude that piperacillin clearance is nonlinear. We have reviewed other articles that either confirm or refute nonlinear kinetics of piperacillin. [1–6] None of these articles provides this type-I error rate information, nor other information about whether or not overfitting was assessed. Hence, we believe that our type-I error calculations are a strength of our manuscript in comparison with other articles on this specific topic. In this context, we presented an abstract at the PAGE conference in Stockholm (June 2019), available via (https://www.page-meeting.org/default.asp?abstract=8894). The main message of this abstract that is that the design of PopPK studies evaluating (non)linear kinetics of piperacillin was far from optimal. We believe our efforts to characterize the type-I error rate are a step into the right direction. Also, type-I error calculations for PopPK studies are highly recommended by the IDeAl consortium. [7]

b) External validation.

Another strength of our manuscript is the fact that we have validated our PK model in a subset of patients different from the ones used for model building, a vital step in model building often lacking in PopPK studies.

Indeed, our model shows a trend towards underprediction but whether or not a trend towards under- or overprediction is also present in the other PopPK studies assessing the (non)linear behavior of piperacillin is unknown since none were validated.

2. Besides those, others can be added: artierial blood samples were collected from patients instead of venous blood samples (why?/);

Answer: There are several reasons for the use of arterial blood samples. First, patients admitted to our ICU have a dedicated arterial bloodline for sampling. It is therefore custom in our ICU (and other ICU's) to use the arterial line for sampling. Second, arterial blood samples for antibiotic concentrations have been used by several other authors. [8,9] Moreover, unlike high extraction ratio drugs such as e.g. propofol, there is no significant arterial-venous difference for piperacillin (personal communication dr Suzanne Parker, University Of Queensland, Brisbane, Australia).

3. The values of AUC predicted by Monte Carlo simulations were not that different for both dosing regimens (Figure 5). Furthermore, free AUC values should have been considered instead of total AUC. Assuming the Clinical and Laboratory Standards Institute susceptibility breakpoint for PIP/TZB of ≤16/4 <mu>g/mL, in all dosing regimens investigated (Figure 5) plasma concentrations were above the MIC for 100% of the dosing interval (% T>MIC), not demonstrating the bias towards PIP intermittent dosing regiments mentioned by the authors.

Answer: We agree with the reviewer. Whether or not free concentrations were used was, by mistake, not stated in our methods section for which apologize. The AUC simulations performed in the manuscript were calculated unbound (free) AUC simulations (AUC_u) assuming a level of protein binding of 30%, which is in accordance with earlier findings. [10] We have now added this to our methods section (lines 206-207). We also changed AUC to

AUC_u in our manuscript, including figure 5. The actual numbers did not change as these values were already (calculated) unbound AUC values.

Further, our study was not intended to provide an answer to the question if the difference in AUC_u between both modes of infusion is of clinical relevance. We believe this question is best answered with a study looking at patient outcome. We merely demonstrate that administering the same dose using different modes of infusion does not necessarily lead to the same antibiotic exposure.

The reviewer further states that 100%$fT_{>MIC}$ was achieved in all simulations. We agree, yet achieving 100% $fT_{>MIC}$ with either intermittent or continuous infusion does not guarantee the same level of bacterial cell kill. In another project, we've specifically looked at preclinical experiments assessing bacterial cell kill with intermittent or prolonged infusion of beta-lactam antibiotics (protocol available via PROSPERO (CRD42018085202). The majority of the experiments with intermittent infusion report a PK/PD target of 40-70% $fT_{>MIC}$ for maximum bacterial cell kill, while continuous infusion experiments most commonly report a C_{ss}/MIC ratio of 4-8 as the preferred PK/PD target for maximal bacterial cell kill. To our knowledge, there is no evidence available that indicates that attaining 100%$fT_{>MIC}$ with intermittent infusion will lead to the same level of bacterial cell kill as 100%$fT_{>MIC}$ achieved with continuous infusion. For example, Alou, *et al*. [11] evaluated the PK/PD target for intermittent and continuous infusion ceftazidime in an *in vitro P*. *aeruginosa* model. For the same PK/PD target (i.e. 100%$fT_{>MIC}$), regrowth was seen in the continuous infusion arm while a 3-$\log_{10}$ kill was seen in the intermittent infusion arm. Of note, the AUC in the intermittent arm was approximately four times higher when compared with the AUC in the continuous infusion arm. Also, Felton, *et al*. [12] document different (up to 3-fold higher) PK/PD targets for the same level of bacterial cell kill with extended as opposed to

intermittent infusion piperacillin. Therefore, we think it is not appropriate to compare intermittent and continuous infusion in terms of the same PK/PD target (in casu 100%$fT_{>MIC}$). Comparing intermittent and continuous infusion in terms of AUC is a validated strategy and was previously done by Firsov and Mattie. [13] This reference was also added in our methods section on line 204-206.

4. Once again, simulations of free plasma concentrations, considering PIP protein binding should have been performed.

Answer: Thank you, we have made the necessary changes (see also answer to question 3).

5. Finally, the authors conclude that other studies should be conducted, appropriately powered and with low type I error, to provide a conclusive evidence of the potential influence on PIP non-linear elimination on critically ill patients treatment, informing that the main goal of the study was not achieved. I would add that the Monte Carlo simulations should consider the investigation of the proper PK/PD index for this drug and no the total AUC proposed in the manuscript. In conclusion, the novelty and the advance in knowledge brought by the study are not clear and seem to be of little clinical significance.

Answer: Our comment in terms of appropriately powered studies with a low type I error rate refers to the fact that, aside from our study, no other study evaluating the (non)linear kinetics of piperacillin mentioned some kind of evaluation or external validation of the study design (see also strengths of our study as a reply the to the first general remark of reviewer #1). We believe we achieved the main goal of our study, given the low likelihood of falsely rejecting

H_0 as demonstrated by the low type-I error rate of our design. It is evident that one can always do better, but we are confident that our approach was certainly not inferior to the approach of other groups.

We do not claim at any point AUC/MIC is the PK/PD index of choice for beta-lactam antibiotics (as stated on line 341 in our discussion). We merely use AUC/MIC to compare two modes of infusion (see also answer to question 3).

As to the question whether our findings are of clinical relevance, we would argue that there are indeed many potential implications. Given the fact that two modes of infusion (i.e. intermittent and continuous infusion) cannot be compared based on one single $\%fT_{>MIC}$, a comparison in terms of AUC is more appropriate (see also reply to question 3).

For the purpose of our systematic review and meta-analysis (registered on PROSPERO, see also answer to question 3), we have selected original preclinical experiments reporting a PK/PD target for beta-lactam antibiotics based on dose finding studies. Second, we calculated the AUC_u/MIC corresponding to the PK/PD target reported in the original experiment (i.e. a PK/PD target of $50\%fT_{>MIC}$ corresponded to an AUC_u/MIC of 356 mg*h/mL). Next, we calculated the $AUC_{u\ 24}$/MIC required to obtain a 1-$\log_{10}$ reduction in CFU/mL in all experiments. A DL random-effects model was used to compare mean (+SD) values of $AUC_{u\ 24}$/MIC for intermittent and continuous infusion experiments. We hypothesized that if continuous infusion has improved killing characteristics when compared to intermittent infusion, then this should be evident from a lower overall antibiotic exposure ($AUC_{u\ 24}$/MIC) required to achieve the same level of bacterial cell kill. This research question has been answered in our review. The first draft has the approval of prof De Waele and prof Lipman and currently awaits approval of the other co-authors.

A difference in AUC_u/MIC is especially relevant for large RCT's comparing intermittent versus prolonged infusion of beta-lactam antibiotics. As you may now, the BLING III (Beta-

Lactam Infusion Group) study, a large, 7000-patient RCT aiming to compare intermittent and continuous infusion piperacillin and meropenem in terms of all-cause mortality on day 90 is currently ongoing. In this study, as in many other RCT's evaluating intermittent versus continuous infusion, the same doses are used in both arms. Our current study clearly demonstrates that administering the same dose with intermittent or continuous infusion does not necessarily lead to the same exposure. We found a higher exposure in the intermittent arm which – when extrapolated to the BLING-III study could impact the results. As we have seen with the experiment by Alou, *et al*. [11] , differences in AUC, although the same $\%fT_{>MIC}$ is achieved, do matter, hence we believe our findings are of direct clinical significance.

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***Highlights**

- Elimination of piperacillin (PIP) is saturable at therapeutic concentrations
- Same dose continuous PIP results in lower exposure compared with intermittent PIP
- Intermittent vs continuous PIP trials may be biased towards intermittent PIP

Saturable elimination of piperacillin in critically ill patients: implications for continuous infusion

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Abstract

Purpose: To evaluate saturation of piperacillin elimination in adult critically ill patients.

Patients and methods: Seventeen adult critically ill patients received continuous and intermittent infusion piperacillin/tazobactam. Piperacillin plasma concentrations (n=217) were analyzed using population pharmacokinetic (PopPK) modeling. Post hoc simulations were performed to evaluate the type I error rate associated with our study. Unseen data was used to validate the final model. The mean error (ME) and root mean squared error (RMSE) were calculated as a measure of bias and imprecision respectively.

Results: A PopPK model with parallel linear and non-linear elimination best fitted our data. The median and 95% confidence intervals for model parameters drug clearance (CL), volume of the central compartment (V), volume of the peripheral compartment (Vp) and intercompartmental clearance (Q) were 9 (7.69 – 11) L/h, 6.18 (4.93 – 11.2) L, 11.17 (7.26 – 12) L and 15.61 (12.66 – 23.8) L/h. The Michaelis-Menten constant (K_m) and the maximum elimination rate for Michaelis-Menten elimination (Vmax) were estimated without population variability in the model to avoid overfitting and inflation of the type I error rate. The population estimates for K_m and V_{max} were 37.09 mg/L and 353.57 mg/h respectively. The ME was -20.8 (95% CI -26.2; -15.4) mg/L while imprecision (RMSE) was 49.2 (95% CI 41.2; 56) mg/L

Conclusion: Piperacillin elimination is (partially) saturable. Moreover, the population estimate for K_m lies within the therapeutic window and therefore saturation of elimination should be accounted for when defining optimum dosing regimens for piperacillin in critically ill patients.

Keywords: piperacillin, pharmacokinetics, critically ill, saturation

Introduction

The ureïdopenicilin piperacillin combined with the beta-lactamase inhibitor tazobactam is frequently used to treat serious infections in critically ill patients [1,2]. In line with other beta-lactam antibiotics, piperacillin has time-dependent killing properties. The time (T) for which the free (*f*) concentration of piperacillin remains above the minimal inhibitory concentration (MIC) is the pharmacokinetic/pharmacodynamic (PK/PD) index of choice, i.e. $\%fT_{>MIC}$ [3].

In the past few years, a wealth of evidence emerged demonstrating that the PK of antimicrobial drugs in critically ill patients is profoundly different from the PK of antimicrobial drugs in healthy volunteers or non-critically ill patients [4]. For beta-lactam antibiotics specifically, changes in volume of distribution and/or changes in renal function in critically ill patients may lead to considerable between- and within-patient PK variability [5]. Previously, a pharmacokinetic point-prevalence study of beta-lactam antibiotics in the ICU reported that 16% of the ICU patients did not achieve the PK/PD target of $50\%fT_{>MIC}$ [6]. As suboptimal antimicrobial use may lead to poor infection outcome, efforts are made to optimize the use of beta-lactam antibiotics [7–9]. Because beta-lactam antibiotics have time-dependent killing properties, prolonging the duration of beta-lactam infusion and thereby extending the time the concentration remains above the MIC, was recently introduced in clinical practice [10,11].

Currently, there is an ongoing debate on whether or not piperacillin elimination is saturable at therapeutic plasma concentrations [12–19]. This mechanism is particularly relevant in the context of the recent introduction of prolonged infusion of beta-lactam antibiotics. Indeed, saturation of piperacillin elimination at therapeutic plasma concentrations implies that, for the total antibiotic exposure in a patient to be the same, a higher daily dose could be necessary when piperacillin is infused continuously as opposed to intermittently. In

clinical practice however, the total daily dose of piperacillin is usually not adapted based on the mode of infusion used [11,20].

The aim of this study was to investigate saturation of piperacillin elimination in critically ill patients receiving both intermittent and continuous infusion piperacillin.

Patients and methods

1. Patients

This prospective interventional study was conducted in the Department of Critical Care Medicine of Ghent University Hospital (Ghent, Belgium). Ethical approval was obtained from the Ghent University Hospital Ethics Committee (registration number 2017/1354). Informed consent was signed by patients or their representatives. Patients were eligible for inclusion if they were admitted to the surgical or medical ICU and received piperacillin/tazobactam (TZP) in continuous infusion. Patients younger than 18 years of age and patients receiving extracorporeal membrane oxygenation (ECMO) or renal replacement therapy (RRT) during antibiotic therapy were excluded from the study. Creatinine clearance was determined by measuring urinary creatinine concentrations from an 8-hour urinary collection using an indwelling urinary catheter. Piperacillin antibiotic concentrations and additional data such as, biochemistry, demographic data, the modified Sequential Organ Failure Assessment score (SOFA) on the day of sampling, the Acute Physiology and Chronic Health Evaluation (APACHE II) score on admission and ICU survival were prospectively recorded via REDCap [21].

2. Administration of piperacillin antibiotic therapy and sampling

All patients received both continuous and intermittent infusion TZP. TZP dosing was as follows: loading dose of 4/0.5 g /30 min immediately followed by a continuous TZP

infusion: (measured creatinine clearance (CL_{CR}) <15 mL/min: 8/1 g /24 h, CL_{CR} 15-29 mL/min: 12/1.5 g /24h and for a $CL_{CR} \geq 30$ mL/min 16/2 g/24h). At the end of the antibiotic course as indicated by the treating physician, after a 3-hour washout period, a short infusion (0.5 h; 4500 g) of TZP was administered. In total, 13 samples were collected from every patient. The first two samples were taken 2 hours prior to and immediately before stopping the continuous infusion. Samples 3-13 were collected immediately before administration of the intermittent infusion and after 5, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes as shown in Figure 1.

3. Bioanalysis of piperacillin plasma concentrations

Arterial blood collected in 4 mL blood tubes (lithium heparin blood collection tubes, BD Vacutainer®, BD Diagnostics, Erembodegem, Belgium) was sent to the core laboratory of the Dept. of Laboratory Medicine at the Ghent University Hospital where they were first stored in a refrigerator at 4°C until they were collected by the toxicology laboratory technicians. Storage at 4°C was never longer than 24 hours. After transferring to an Eppendorf tube, plasma samples were centrifuged at 16162x*g* for 8 minutes (Microfuge 16, Beckman Coulter, Brea, California). Immediately afterwards, the plasma samples were stored at -20˚C until analysis. All samples were analyzed within 1 week. The plasma concentration of piperacillin was determined by ultra-performance liquid chromatography tandem mass spectrometry (UPLC – MS/MS). Tazobactam concentrations were not analyzed in this study. The lower limit of quantification (LLOQ) for piperacillin was 1.09 mg/L, the within-run assay imprecision at LLOQ level was 3.7 %CV and the between-run assay imprecision at the LLOQ level was 8.1 %CV [22].

4. Population pharmacokinetic model building

Piperacillin concentration-time data were analyzed using Pmetrics (version 1.5.2; Laboratory of Applied Pharmacokinetics, Los Angeles, CA, USA), an R-based software program for non-parametric and parametric pharmacokinetic-pharmacodynamic population and individual modelling and simulation. We used the non-parametric adaptive grid (NPAG) algorithm to build a PopPK model for piperacillin administered via continuous and intermittent infusion [23]. A digital Fortran compiler was used (Gfortran version 6.1; Free software foundation, Inc. Boston, MA, USA) and the runs were executed using R (version 3.5.1; The R Foundation for Statistical Computing. Vienna, Austria) and RStudio (version 1.1.383; RStudio, Inc. Boston, MA, USA). One- and two compartment models were fitted to the data using subroutines from the Pmetrics library. Modeling concentration-time data with both linear, parallel linear/Michaelis-Menten and Michaelis-Menten drug clearance was attempted. Subsequently, the statistical error model with the best fit was selected and a covariate model was developed. Covariates *a priori* considered for inclusion in the model were: measured creatinine clearance, estimated creatinine clearance (Cockroft-Gault formula), estimated glomerular filtration rate (Modification of Diet in Renal Disease (MDRD) formula), body weight, age, SOFA score and albumin, based on prior knowledge and biological plausibility [4,24–27]. Body weight was included as a primary covariate on all model parameters, except for K_m and V_{max}, according to the allometric power model [28].

(1) $P\ \theta_i = TVP\theta_1*(WEIGHT/70)**power$ Eq. 1

Where $P\ \theta_i$ is the individual parameter value, $TVP\theta_1$ is the parameter value for a typical adult with a body weight of 70kg, and power is an allometric exponent fixed to 0.75 for CL and Q and fixed to 1 for V and Vp. As an initial step, covariates measured creatinine, estimated creatinine clearance via Cockroft-Gault formula and estimated glomerular filtration rate using the MDRD formula were tested on the CL parameter as this is biologically plausible.

However, only one of these was retained as correlated variables may lead to collinearity and inflation of the parameter's standard error [29]. In a next step, forward selection and backward elimination using the PMstep function in Pmetrics was used to assess the relationship between covariates and model parameters. The log likelihood ratio test (LRT) and the Akaike information criterion (AIC) were considered during model building. More specifically, a difference of 3.84 in the log likelihood was considered significant at the 5% level when performing the likelihood ratio test for comparing nested models. Estimated parameters are reported as mean, percent coefficient of variation (%CV) and median with interquartile range (IQR). The %CV is reported as a measure of between-subject variability in the model parameters. 95% Confidence intervals were estimated *via* a non-parametric bootstrap (n=1000) and quantify the uncertainty on the parameter estimates.

5. Pharmacokinetic model diagnostics

The PopPK model was assessed by visual evaluation of the goodness of fit of the observed versus *a posteriori* predicted plots and the coefficient of determination of the linear regression of the observed-predicted values (r^2 close to 1, intercept close to 0) from each run. The predictive performance was assessed on mean prediction error (bias) and the mean bias-adjusted square prediction error (imprecision) of the population predictions.

Internal model validation consisted of a visual predictive check (VPC) plot. The VPC (n=10.000) was performed by overlaying the 95% CI of the simulated profiles for 0.05, 0.5 and 0.95 quantiles with the corresponding quantiles of the observed data.

For external model validation, the final model population parameter distributions were used to predict concentrations for an independent validation dataset. We refer to Dhaese, *et al* [30] for a detailed description of this validation dataset. Prediction errors were evaluated based on the absolute bias (ME) and imprecision (MSE) as described in equation 2 and 3:

(2) Absolute bias[$\hat{\theta}$] (ME) = E[$\hat{\theta} - \theta$] Eq.2

(3) Absolute imprecision[$\hat{\theta}$] (MSE) = E[$(\hat{\theta} - \theta)^2$] Eq.3

Where $\hat{\theta}$ is the predicted piperacillin concentration and θ is the observed concentration. The root mean square prediction error (RMSE) was calculated by taking the square root of MSE.

6. Comparative AUC_u simulations for intermittent and continuous infusion dosing regimens

Monte Carlo simulations (n=1000) were performed with the final PopPK model to compare the unbound (u) area under the curve (AUC_u) as a measure of total (unbound) drug exposure between intermittent and continuous infusion dosing regimens. Using AUC as a basis to compare intermittent and continuous infusion of beta-lactam antibiotics was previously reported by Firsov and Mattie [31]. Free piperacillin concentrations were calculated assuming a 30% level of protein binding in accordance with previous findings [32]. Four different scenarios were evaluated; i.e. a daily dose of 12/1.5g TZP for a patient with a measured CL_{CR} of 20mL/min, 16/2g TZP for a patient with a measured CL_{CR} of 70mL/min, 16/2g TZP for a patient with a measured CL_{CR} of 130mL/min and 16/2g TZP for a patient with a measured CL_{CR} of 200mL/min. The body weight for all patients was fixed at 70kg. For each of these four scenarios, both intermittent and continuous infusion dosing regimens were simulated and compared. The AUC_u was calculated using linear trapezoidal approximation. A 24-hour interval for AUC_u calculation was chosen after six doses for intermittent infusion and one bolus and five maintenance doses for continuous infusion.

7. *Post hoc* estimation of type I error rate

A type I error rate analysis was performed to evaluate the probability to reject the null-hypothesis (H_0) in favor of the alternative hypothesis (H_1) given that it is true, where H_0 =

piperacillin kinetics are best described by linear elimination and H_1= piperacillin kinetics are best described by non-linear elimination. [27]

In short, we simulated concentrations for 17 patients according to the design of this study (drug administration, blood sampling, etc.). For this, the PopPK model by Landersdorfer, *et al* [12] served as the H_1, i.e. piperacillin PKs are non-linear and elimination is characterized by a parallel first-order and Michaelis-Menten process. The H_0 was simulated by fixing the V_{max} estimate in the model by Landersdorfer to zero, i.e. removing the non-linear component in piperacillin elimination. This process was repeated 5000 times, resulting in 10,000 simulated datasets. All simulated datasets were fitted with a two-compartmental model with linear elimination and a two-compartmental model with parallel linear and Michaelis-Menten elimination. Both models were compared using the LRT according to equation 4.

(4) $LRT = 2*(LL_c - LL_r)$ Eq. 4

where LL_c is the log likelihood (LL) for the more complex model and LL_r is the LL for the reduced model. The difference in the number of parameters between both models was 4 when between-subject variability was included in the estimation of K_m and V_{max} and was 2 otherwise. When considering the 5% level of significance, the critical values from the chi-square distribution were 9.49 and 5.99, respectively.

The type I error rate was calculated from the number of times the complex model was declared superior over the reduced model for the simulated datasets according to the H_0.

8. Statistical analysis

All statistical analyses were performed using R and RStudio. Continuous data are presented as median (interquartile range). Categorical data are presented as counts (%).

Results

1. Patients and samples

In total, 17 patients were included, and 221 samples were collected (Table 1). All patients were enrolled between 5/2/2018 and 18/10/2018. Samples 5-7 were lost for patient 13 and sample 8 was lost for patient 15, therefore only 217 samples were analyzed and used for PK model building. The focus of infection was respiratory in 11 patients, abdominal in 5 patients and bacteremia in 1 patient.

2. Pharmacokinetic model building and model diagnostics

Table 2 summarizes the log-likelihood values, the coefficients of determination (r^2 values), the AIC's and the predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten models (without covariates). Comparison of the coefficient of determination, the bias, imprecision and AIC indicated that the model with parallel linear and Michaelis-Menten kinetics was superior compared to both a model with linear elimination and a model with Michaelis-Menten elimination alone (Table 2).

Including measured creatinine clearance (mCRCL) normalized to 100 mL/min as opposed to estimated creatinine clearance using the Cockroft-Gault or the estimated glomerular filtration rate using the MDRD formula provided the model with the lowest AIC value (Table 3). Forward selection and backward elimination further revealed a relationship between albumin and clearance. However, when including albumin as a covariate on CL, no model improvement in terms of ΔAIC or LRT was noted, hence albumin was not retained as a covariate in the final model.

The final model was described as:

(5) $CL = TVCL*(mCL_{CR}/100) *(WEIGHT/70)**0.75$ Eq. 5

(6) $V = TVV*(WEIGHT/70)$ Eq. 6

(7) $Vp = TVVp * (WEIGHT/70)$ Eq. 7

(8) $Q = TVQ^* (WEIGHT/70)^{**}0.75$ Eq. 8

where CL is piperacillin clearance, V is volume of distribution of the central compartment, Vp is volume of distribution of the peripheral compartment and Q is the intercompartmental clearance. TVCL refers to the population typical piperacillin clearance for a 70-kg patient with a mCL_{CR} of 100 mL/min, TVV and TVVp refer to the population typical volume of distribution of the central, respectively the peripheral compartment for a 70-kg patient.

The mean, %CV, median (IQR) and %95 CI around the median for the population parameter estimates are listed in Table 4. The typical value for K_m and V_{max} was 37.09 mg/L and 353.57 mg/h respectively.

Between-subject variability was not estimated on K_m and V_{max} as this resulted in an over-parameterized model and an unacceptable inflation of the type I error rate (for further details see the section "*Post hoc* estimation of type I error rate"). Based on the diagnostic plots, the γ multiplicative error model was selected for modelling assay variance. In all model-building runs, each observation was weighted by $1/ (\gamma \times SD^2)$. We set γ equal to 1 initially and allowed Pmetrics to fit the value for the population. The final-cycle γ value was 1.26, indicating some additional process noise. The formula for the γ error model is error= γ*SD where SD is the standard deviation of each observation. SD is modeled by equation 9 and was based on earlier validation work by Carlier, *et al* [33].

(9) $SD = 2 + 0.1x\ C$ Eq. 9

where C is the concentration of piperacillin.

The *a posteriori* individual and population predicted versus observed plots and the VPC plots are shown in figure 2 and 3 respectively. The results of the Shapiro-Wilk test of normality for the NPDE indicated no violation of normality (p=.195).

The final PopPK models showed a bias (ME) in predicting serum concentrations from the validation dataset of -20.8 (95% CI -26.2 ; -15.4) mg/L while imprecision (RMSE) was 49.2 (95% CI 41.2 ; 56) mg/L. The Bland-Altman plot is shown in figure 4.

3. Comparative AUC_u simulations for intermittent and continuous infusion dosing regimens

In all four scenarios, patients receiving continuous infusion had lower AUC_u values when compared to simulated patients receiving the same dose *via* intermittent infusion (figure 5).

4. *Post hoc* estimation of type I error rate

If the between-subject variability was estimated for all model parameters, the type I error rate was 47.9%. If the between-subject variability was estimated for CL, Q, V and Vp and not estimated for K_m and V_{max}, the type I error rate was reduced to 6.6%.

Discussion

A PopPK model with parallel linear and Michaelis-Menten elimination of piperacillin best described this data, collected from 17 critically ill patients receiving both intermittent and continuous infusion piperacillin/tazobactam. These findings are in agreement with previous studies in healthy volunteers and non-critically ill patients [12,13,17] and in disagreement with other studies in healthy volunteers and critically ill patients [14,30,34].

Renal excretion of piperacillin is the major pathway of elimination. Approximately 74-89% of the administered dose of piperacillin is eliminated from the body by renal excretion [2,35]. More specifically, Tjandramaga, *et al*. [35] reported that 56-73% of the renally cleared piperacillin is eliminated through tubular secretion, which is a saturable process.

V_{max} is the maximum elimination rate for Michaelis-Menten elimination and the drug concentration at which the elimination rate is half of the maximum elimination rate is called the Michaelis-Menten constant or K_m. Whether or not non-linear elimination of a drug is clinically relevant depends on the value of V_{max} and K_m. Non-linear elimination is a clinically relevant process if saturation occurs at therapeutic concentrations (i.e. K_m within the therapeutic window) and if V_{max} is high relative to CL, indicating a substantial contribution of the non-linear elimination process to the total body clearance. It is postulated that the non-linear elimination pathway should contribute to at least 20% of the total body clearance for it to be clinically relevant [36]. If K_m is very high, then saturation occurs but not at relevant plasma concentrations and it will therefore have no impact on the optimal dosing regimen [12]. Other researchers have reported K_m estimates of 36.1 mg/L [12], 47.9 mg/L [13] and 90.13 mg/L [17], all well in the range of therapeutic piperacillin plasma concentrations and in line with our estimate of 37.09 mg/L.

The implications of these findings remain to be determined. Several institutions recently moved towards prolonged infusion of beta-lactam antibiotics yet conclusive evidence in favor of prolonged infusion is lacking and new clinical trials are in the pipeline [10,11,20,37]. Saturation of piperacillin elimination at therapeutic plasma concentrations is of particular relevance when randomized clinical trials compare intermittent versus continuous infusion piperacillin. Indeed, if saturation of piperacillin elimination occurs at therapeutic concentrations, clinical trials comparing the same daily dose of intermittent and continuous infusion piperacillin may unwillingly introduce a bias towards intermittent infusion as patients receiving the same daily dose of piperacillin *via* intermittent infusion may have a higher total antibiotic exposure when compared to patients receiving the same dose of piperacillin *via* continuous infusion as is demonstrated in the $AUC_{u\ 24}$ calculations using the final PopPK model (figure 5). While AUC_u/MIC may not be the PD index of choice for beta-

lactam antibiotics, the phenomenon of non-linear kinetics may impact antibiotic concentrations and indirectly also other PD indices such as $T_{>MIC}$. This study focused on piperacillin but tubular secretion of other beta-lactam antibiotics such as amoxicillin, oxacillin, flucoxacillin, cefazolin and cefuroxime has been reported as well [38,39].

When performing hypothesis testing and PK model selection, control of the type I error rate is pivotal to avoid false positive conclusions. Inflation of the type I error rate is expected when dealing with (very) small datasets [40,41]. In this study, including the between-subject variability on K_m and V_{max} resulted in an over-parameterized model and an unacceptable type I error rate (for further details see the section "*Post hoc* estimation of the type I error rate"). Therefore, the between-subject variability for K_m and V_{max} was not estimated. As few piperacillin population PK studies incorporate type I error calculations, it is difficult to determine how our findings with regard to the non-linear kinetics of piperacillin relate to the findings of other studies.

This study has several limitations. While our primary goal was to detect non-linear elimination of piperacillin with a low probability of falsely rejecting H_0, the between-subject variability was not estimated on K_m and V_{max} as this led to an unacceptable type I error. Determining urinary concentrations of renally eliminated drugs is helpful when non-linear kinetics are expected, however, in this study, piperacillin concentrations were not measured in the urine and no distinction could be made between the renal and non-renal clearance of piperacillin. The validation results indicate that the final model has a bias towards underpredicting antibiotic concentrations. While no bias is to be preferred, in case of underprediction, physicians may be inclined to increase the dose or dosing frequency. Given the low toxicity of beta-lactam antibiotics and the important risk of underdosing in ICU patients, models that underpredict concentrations of beta-lactam antibiotics are usually preferred over models that have bias towards overprediction [42]. Additionally, the sequence

of the infusion modes never changed and all patients received continuous infusion first, followed by intermittent infusion. Hence, a trend in piperacillin clearance over time could not be excluded.

In conclusion, piperacillin elimination was best described by a PopPK model incorporating parallel linear and Michaelis-Menten elimination. Nevertheless, in literature conflicting evidence is found on the importance of non-linear elimination for piperacillin PK. Non-informative study designs, and statistical inference based on over-parameterized models likely contribute to these conflicting findings. Future studies, appropriately powered and with a low type I error rate, should be conducted to provide conclusive evidence on the potential influence of non-linear elimination for piperacillin PK in critically ill patients.

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Jan De Waele has been consultant for Accelerate Diagnostics, Bayer Healthcare, MSD and Pfizer

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Captions and legends of tables and figures

Tables

Table 1: Patient characteristics, laboratory data and infection characteristics

Table 2: Predictive performance of linear and non-linear piperacillin population PK models

Predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten model. R^2 is the coefficient of determination for the best-fit linear regression for the predicted-observed plot. LL is the log likelihood estimate. AIC is the Akaike information criterion. L = linear, MM= Michaelis-Menten.

Table 3: Predictive performance of piperacillin population PK models incorporating renal clearance as a covariate

Predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten model. R^2 is the coefficient of determination for the best-fit linear regression for the predicted-observed plot. LL is the log likelihood estimate. AIC is the Akaike information criterion. mCL_{CR} = measured creatinine clearance, GaG = estimated creatinine clearance using the Cockroft-Gault formula, MDRD = estimated glomerular filtration rate using the MDRD formula.

Table 4: Mean, %CV, median (IQR), and 95%CI parameter estimates for the final PopPK model

Figures

Figure 1: Administration of piperacillin and timing of sampling

Figure 2: The population predicted versus observed concentrations (left) and the individual predicted versus observed concentrations (right) diagnostic plots for the final PK model. The dashed line is the line of unity and the solid line is the line of the best linear fit.

Figure 3: Visual predictive check plot of piperacillin plasma concentrations (log10 scale) vs. time for the final PopPK model. Black dots represent observed data, solid lines represent quantiles of the observed data and dashed lines represent quantiles of the simulated data.

Figure 4: Bland-Altman plot for comparison of predicted versus observed piperacillin concentrations from a validation dataset. The blue line represents the mean difference in concentrations. Red lines are mean-1.96*SD (lower line) and mean+1.96*SD (upper line).

Figure 5: Simulations of mean (sd) AUC_u values and time-concentration curves for a total daily dose of 12/1g PIP (upper graph) or 16g PIP via intermittent (left) or continuous (right) infusion for a patient with a body weight of 70kg and a measured CL_{CR} of respectively 20, 70, 130 and 200mL/min. AUC_u values were calculated for a 24-hour interval after the sixth dose.

Table 1

Table 1: Patient characteristics, laboratory data and infection characteristics

Patient characteristics			**Median (IQR) or count (%)**
Male, n (%)			11 (64.7%)
Age in years, median (IQR)			64 (51-70)
Weight in kg, median (IQR)			75 (69-80)
APACHE II, median (IQR)			20 (14-24)
SOFA, median (IQR)			7 (5-8)
Duration of TZP therapy in days, median (IQR)			5.8 (4.3-6.8)
Mechanical ventilation during TZP therapy, n (%)			13 (76.5%)
Vasopressive therapy during TZP therapy, n (%)			6 (35.3%)
ICU length of stay in days, median (IQR)			17.9 (14.1-31.5)
ICU survival, n (%)			15 (88.2%)
Albumin in g/L			**Median (IQR)**
72h prior to sampling			26.5 (22-29.5)
48h prior to sampling			26 (21-27.5)
24h prior to sampling			26.5 (22.8-30.3)
Day of sampling			27 (21.5-30.5)
24h post sampling			27 (21.5-30.8)
Timing	**Estimated creatinine clearance (Cockroft-Gault) in mL/min Median (IQR)**	**Estimated creatinine clearance (MDRD) in mL/min Median (IQR)**	**Measured creatinine clearance (mCRCL) in mL/min Median (IQR)**
72h prior to sampling	82.9 (52.3-147.3)	97.9 (49.8-145.6)	70 (30-138)
48h prior to sampling	85.2 (41.1-139.2)	92.9 (36.5-140.9)	49.5 (16.8-141.5)
24h prior to sampling	84.7 (39.9-119.3)	70.3 (59.8-78.6)	87 (43-120)
Day of sampling	86.1 (40.8-139.2)	101.1 (35.2-140.9)	82 (32.5-98)
24h post	100.1 (48.3-139.2)	72.9 (60.6-81.5)	83.5 (36-149.3)

sampling			

Table 2

Table 2: Predictive performance of linear and non-linear piperacillin population PK models

		Linear regression of observed-predicted for each patient					
Model	-2LL	Intercept	Slope	r^2	Bias	Imprecision	AIC
L	1842	3.73	0.98	0.977	-0.078	0.995	1852
L/MM	1748	5.33	0.96	0.975	-0.147	1.31	1797
MM	2197	38.9	0.933	0.647	-0.457	0.779	2207

Predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten model. R^2 is the coefficient of determination for the best-fit linear regression for the predicted-observed plot. LL is the log likelihood estimate. AIC is the Akaike information criterion. L = linear, MM= Michaelis-Menten.

Table 3

Table 3: Predictive performance of piperacillin population PK models incorporating renal clearance as a covariate

		Linear regression of observed-predicted for each patient					
Model	-2LL	Intercept	Slope	r^2	Bias	Imprecision	AIC
mCL_{CR}	1796	4.87	0.97	0.986	-0.136	1.25	1806
GaG	1805	6.08	0.959	0.97	-0.172	1.29	1815
MDRD	1904	5.5	0.98	0.962	-0.12	0.96	1915

Predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten model. R^2 is the coefficient of determination for the best-fit linear regression for the predicted-observed plot. LL is the log likelihood estimate. AIC is the Akaike information criterion. mCL_{CR} = measured creatinine clearance, GaG = estimated creatinine clearance using the Cockroft-Gault formula, MDRD = estimated creatinine clearance using the MDRD formula.

Table 4

Table 4: Mean, %CV, median (IQR), and 95%CI parameter estimates for the final PopPK model

Parameter	**Mean**	**%CV**	**Median (IQR)**	**95% CI around the median**
V (L)	9.74	87.27%	6.18 (5.76 – 6.52)	4.93 – 11.2
CL (L/h)	9.29	26.19%	9 (8.68 – 9.43)	7.69 – 11
Q (L/h)	21.47	59.81%	15.61 (13.38 – 20.29)	12.66 – 23.8
Vp (L)	9.8	34.11%	11.17 (10.7 – 11.69)	7.26 – 12

Figure 1

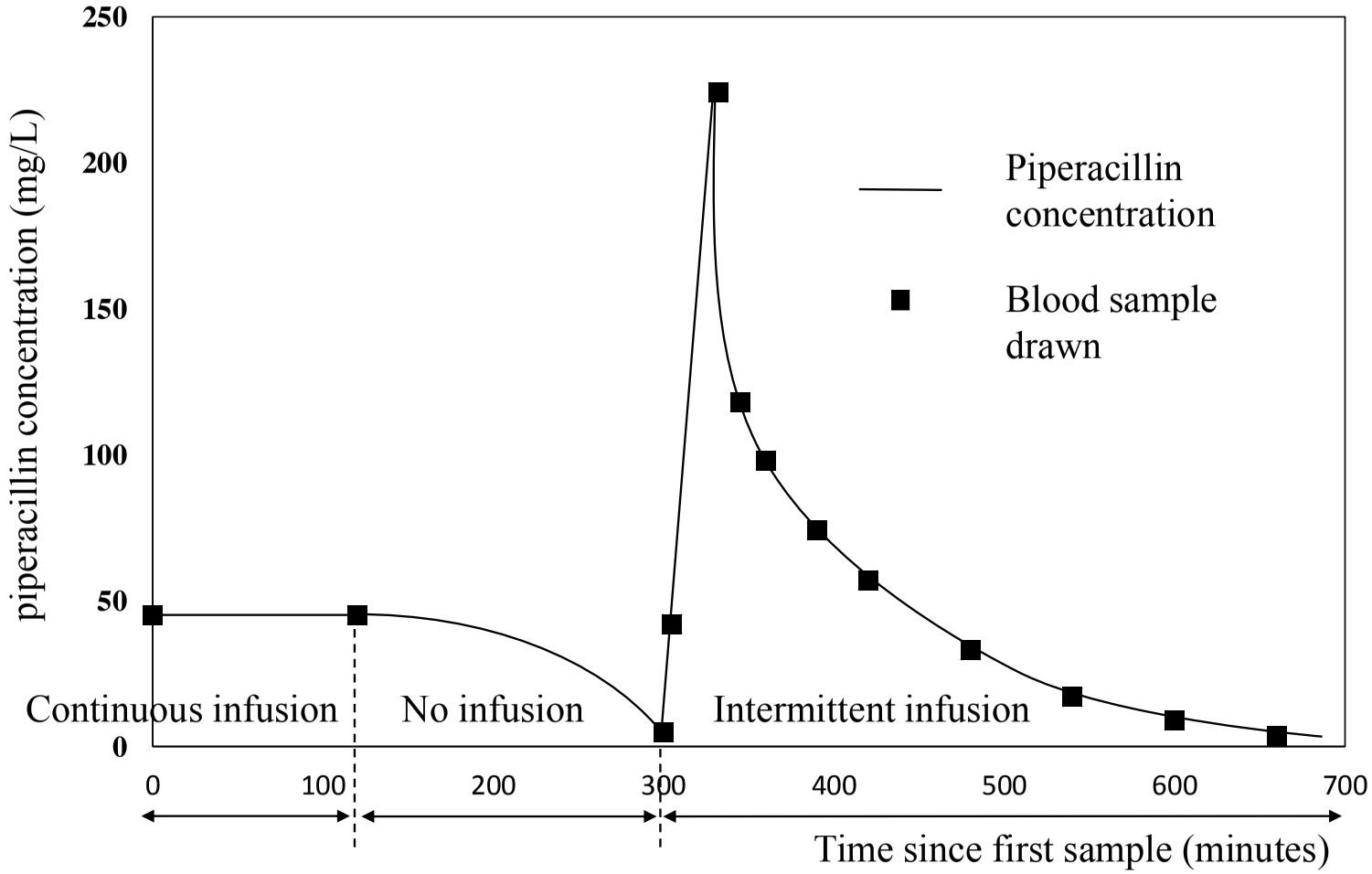
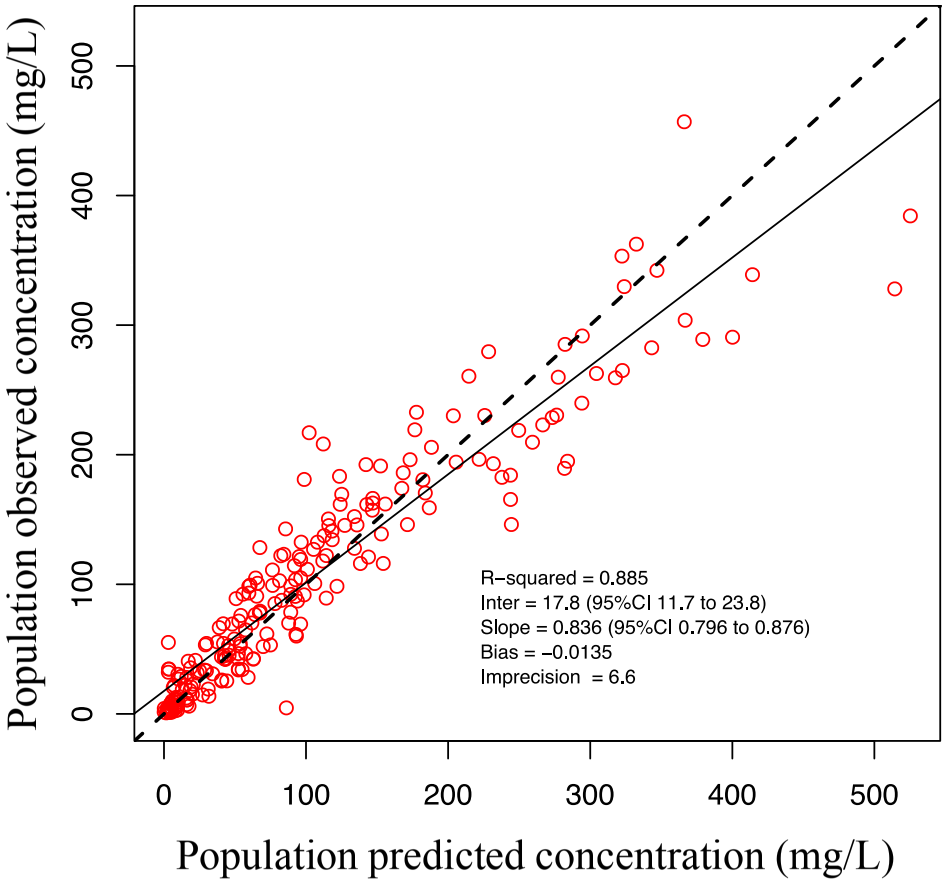

250
200
150
100
50
0
piperacillin concentration (mg/L)
Piperacillin concentration
Blood sample drawn
Continuous infusion
No infusion
Intermittent infusion
0
100
200
300
400
500
600
700
Time since first sample (minutes)

Figure 2


Population observed concentration (mg/L)
0
100
200
300
400
500
Population predicted concentration (mg/L)
R–squared = 0.885
Inter = 17.8 (95%CI 11.7 to 23.8)
Slope = 0.836 (95%CI 0.796 to 0.876)
Bias = –0.0135
Imprecision = 6.6

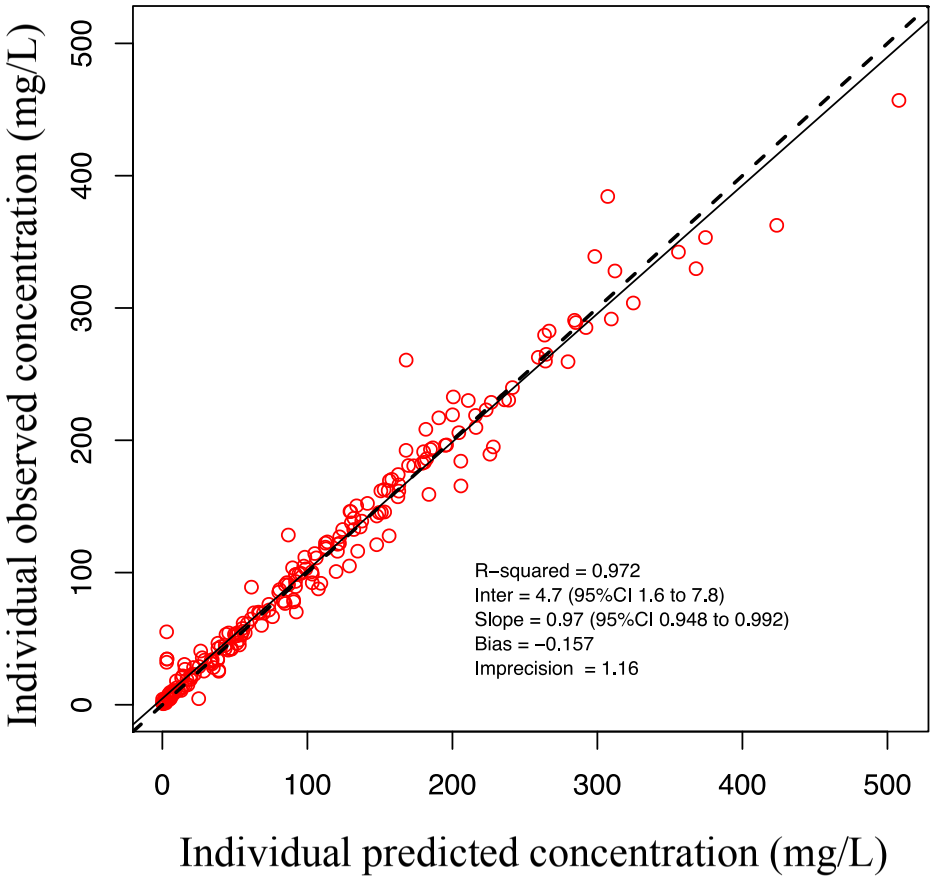

Individual observed concentration (mg/L)
0
100
200
300
400
500
Individual predicted concentration (mg/L)
R–squared = 0.972
Inter = 4.7 (95%CI 1.6 to 7.8)
Slope = 0.97 (95%CI 0.948 to 0.992)
Bias = –0.157
Imprecision = 1.16

Figure 3

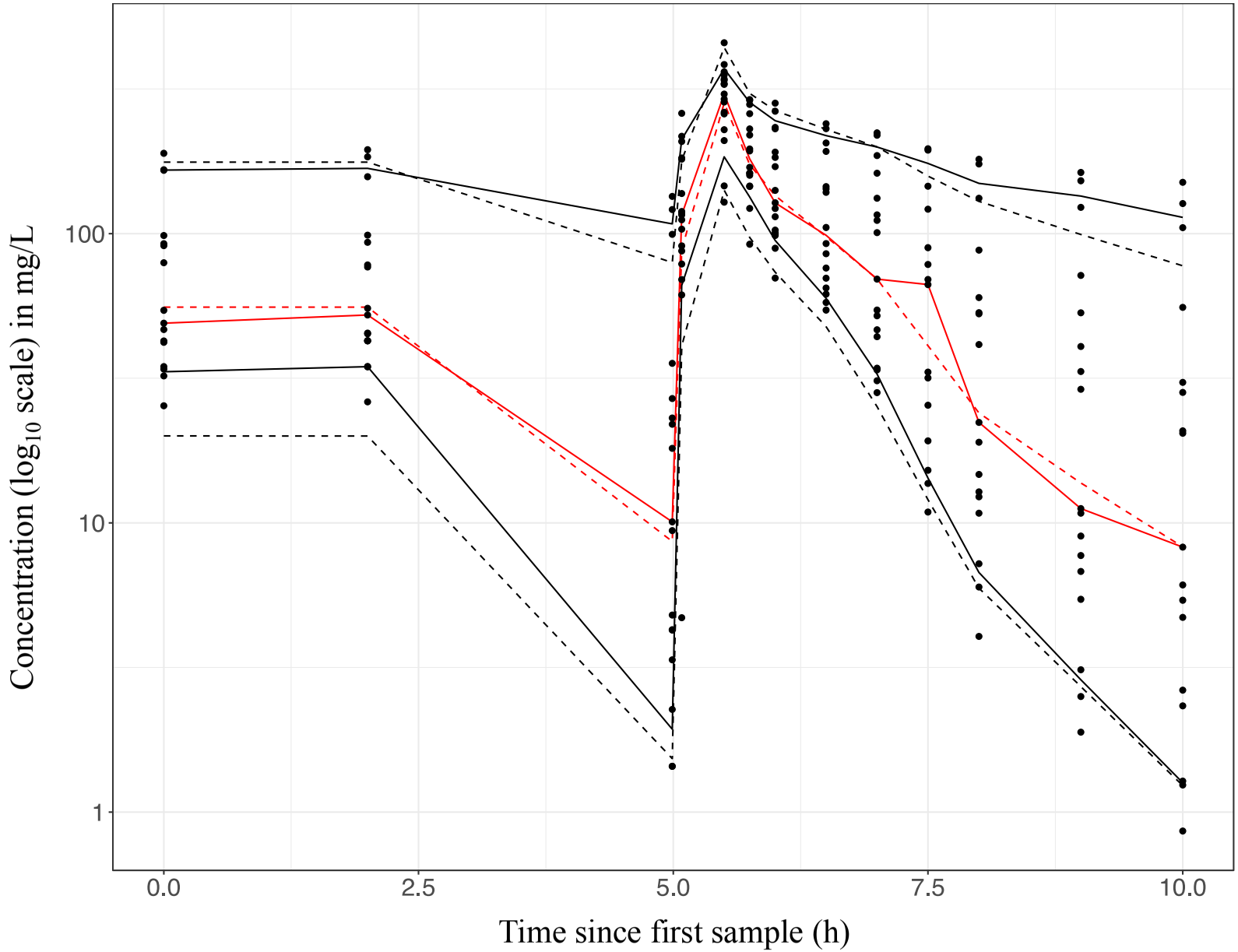

Concentration (log_{10} scale) in mg/L
100
10
1
0.0
2.5
5.0
7.5
10.0
Time since first sample (h)

Figure 4

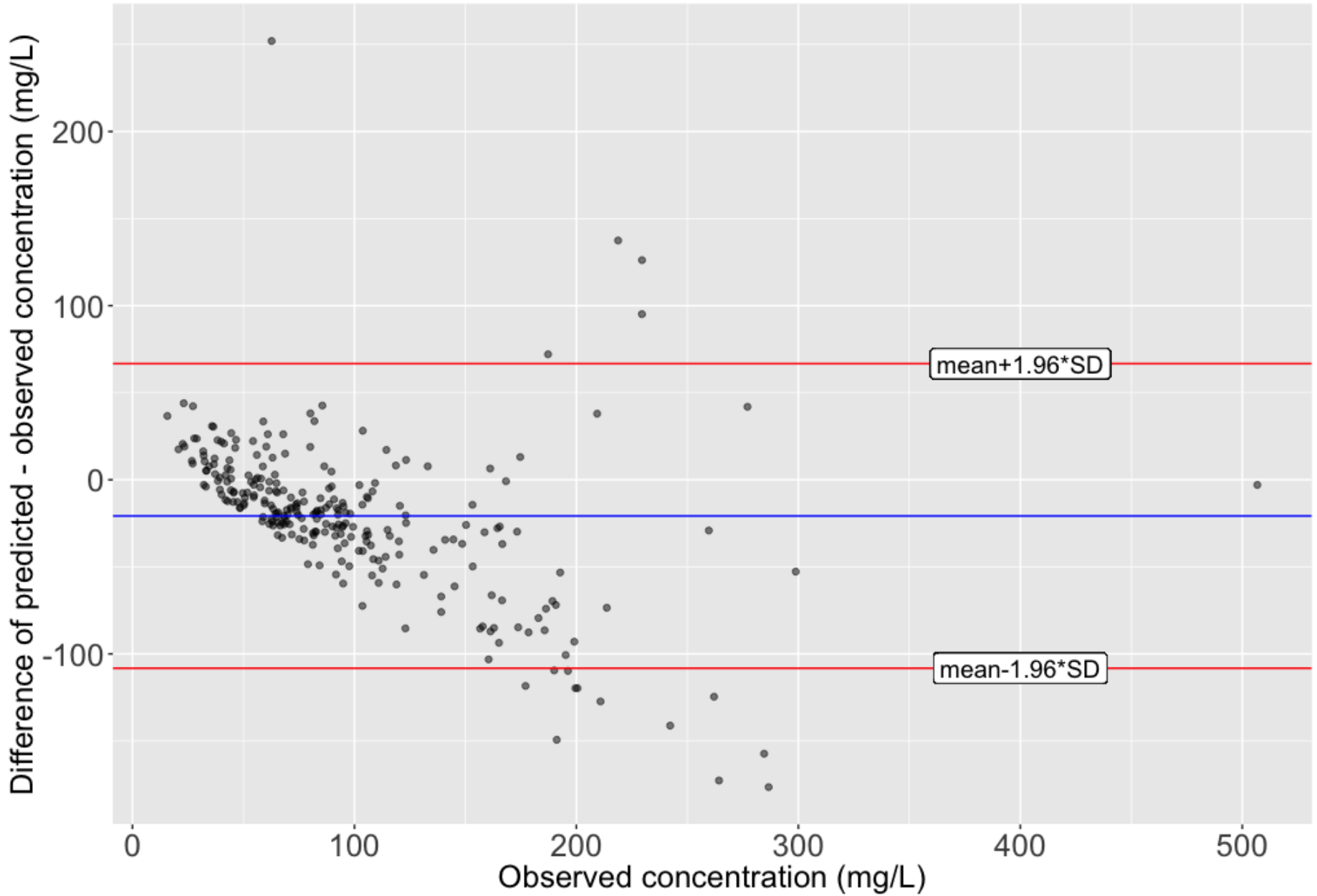
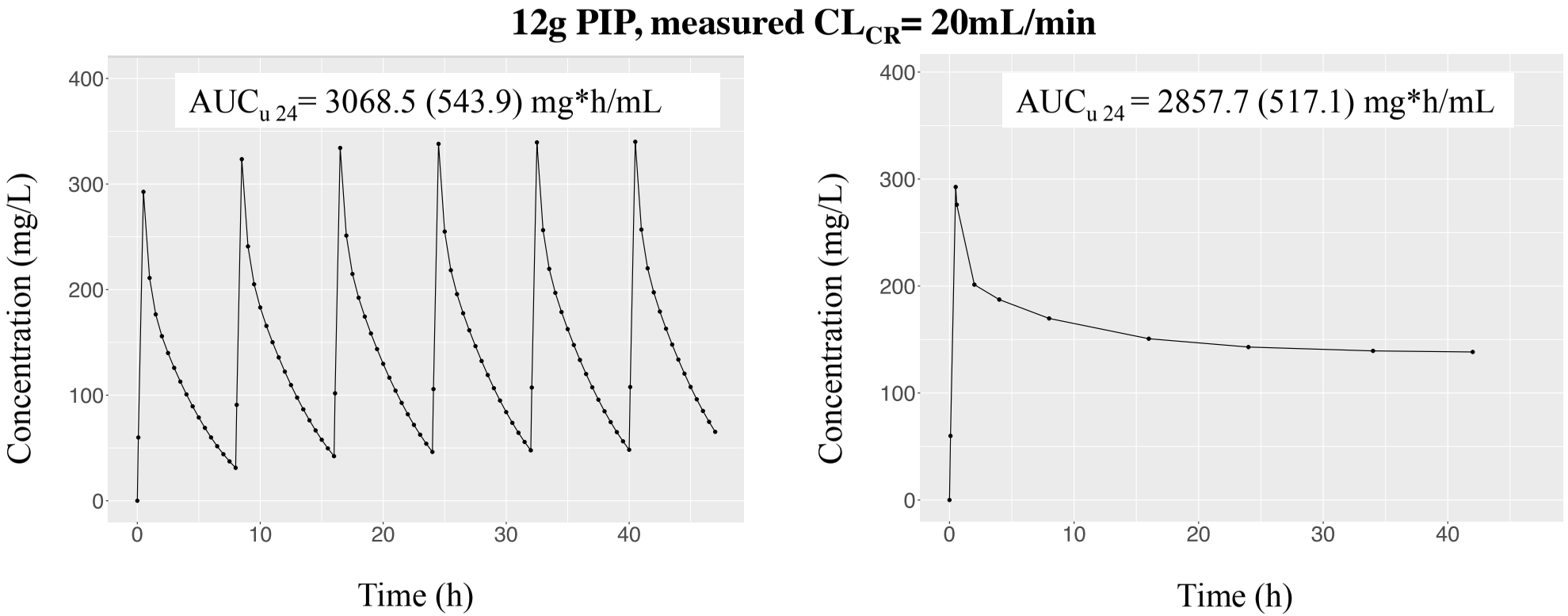
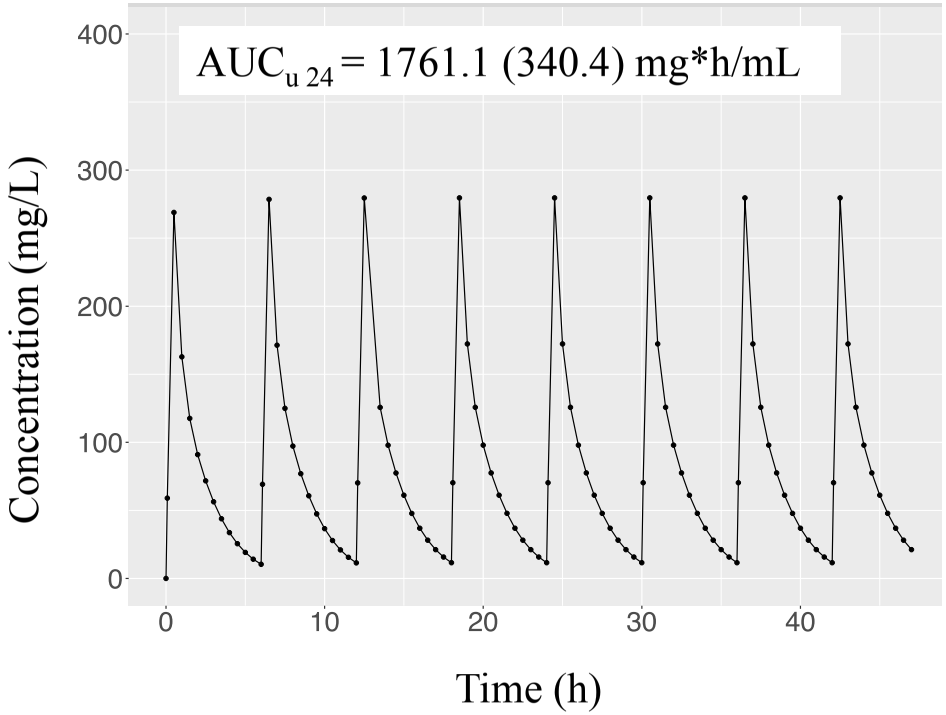
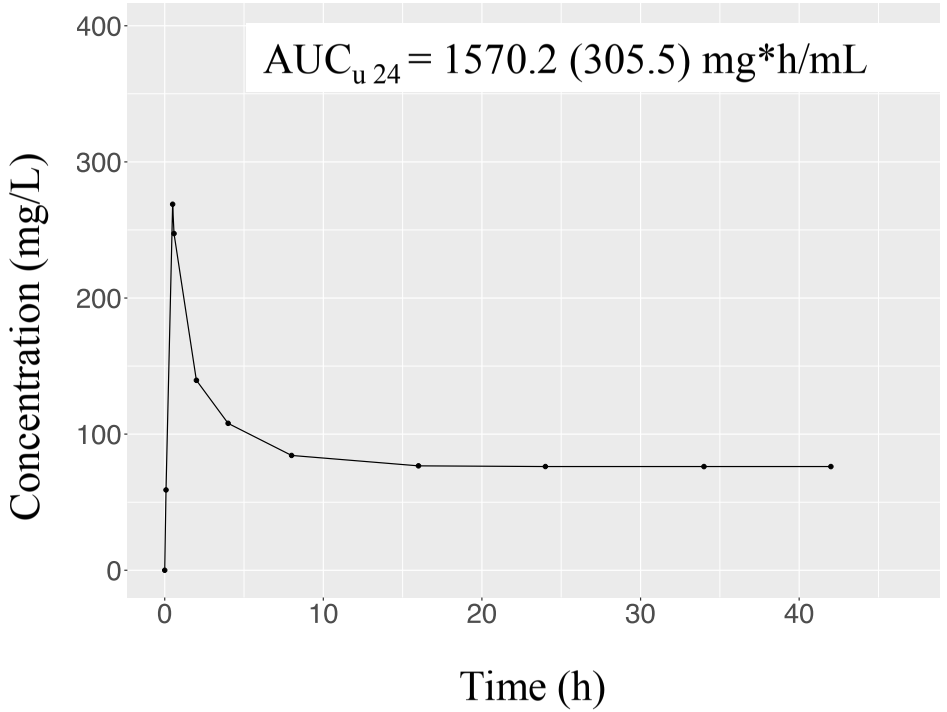

Difference of predicted - observed concentration (mg/L)
200
100
0
-100
mean+1.96*SD
mean-1.96*SD
0
100
200
300
400
500
Observed concentration (mg/L)

Figure 5

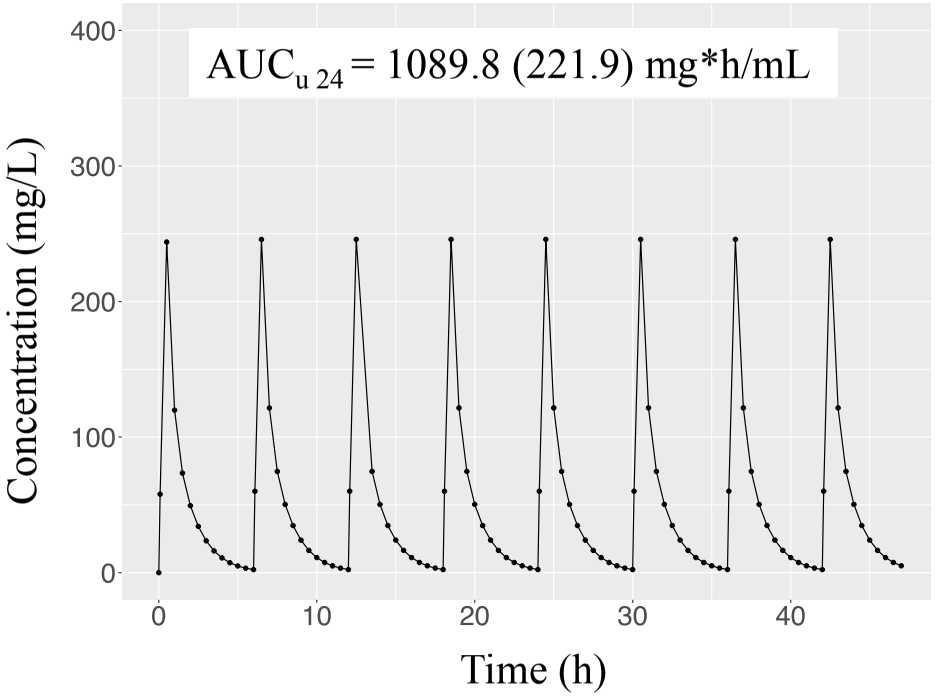

12g PIP, measured CL_{CR}= 20mL/min
$AUC_{u\ 24}$= 3068.5 (543.9) mg*h/mL
$AUC_{u\ 24}$ = 2857.7 (517.1) mg*h/mL
Concentration (mg/L)
Time (h)
0
100
200
300
400
10
20
30
40

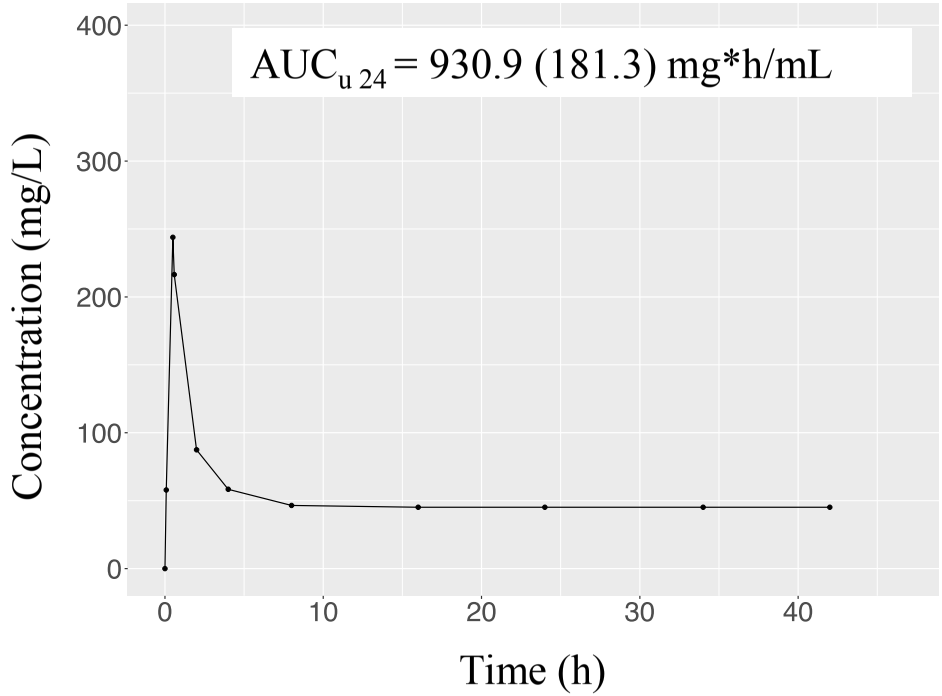
16g PIP, measured CL_{CR}= 70mL/min



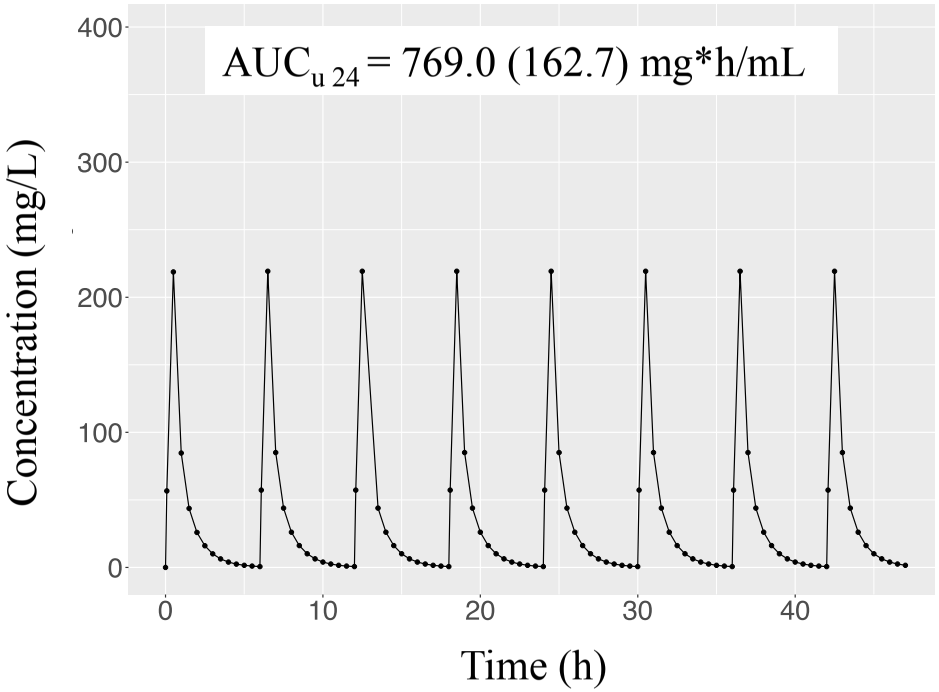


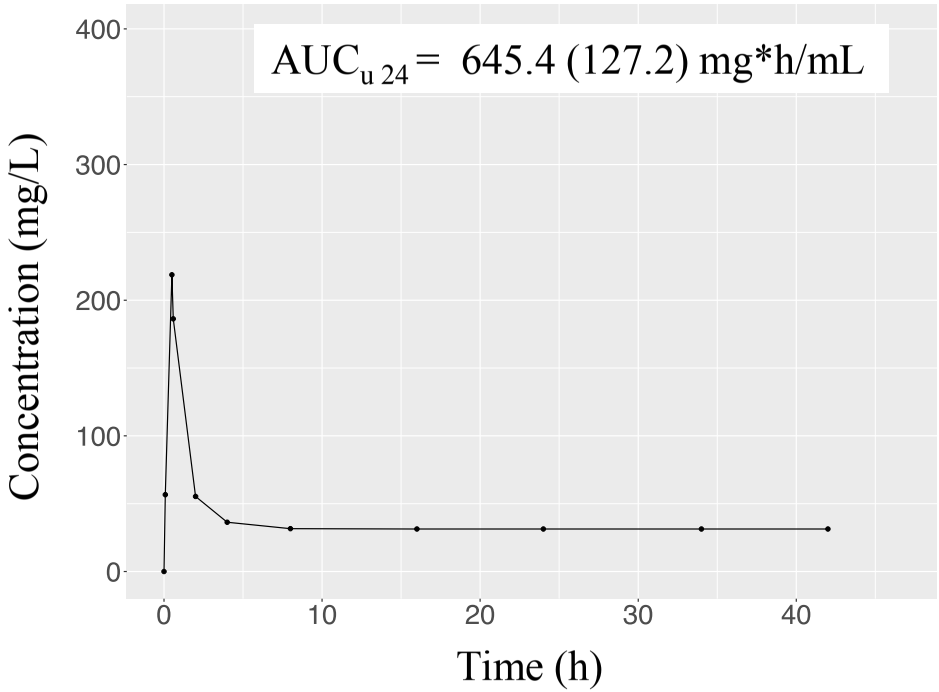
16g PIP, measured CL_{CR}= 130mL/min





16g PIP, measured CL_{CR}= 200mL/min





Saturable elimination of piperacillin in critically ill patients: implications for continuous infusion

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Abstract

Purpose: To evaluate saturation of piperacillin elimination in adult critically ill patients.

Patients and methods: Seventeen adult critically ill patients received continuous and intermittent infusion piperacillin/tazobactam. Piperacillin plasma concentrations (n=217) were analyzed using population pharmacokinetic (PopPK) modeling. Post hoc simulations were performed to evaluate the type I error rate associated with our study. Unseen data was used to validate the final model. The mean error (ME) and root mean squared error (RMSE) were calculated as a measure of bias and imprecision respectively.

Results: A PopPK model with parallel linear and non-linear elimination best fitted our data. The median and 95% confidence intervals for model parameters drug clearance (CL), volume of the central compartment (V), volume of the peripheral compartment (Vp) and intercompartmental clearance (Q) were 9 (7.69 – 11) L/h, 6.18 (4.93 – 11.2) L, 11.17 (7.26 – 12) L and 15.61 (12.66 – 23.8) L/h. The Michaelis-Menten constant (K_m) and the maximum elimination rate for Michaelis-Menten elimination (Vmax) were estimated without population variability in the model to avoid overfitting and inflation of the type I error rate. The population estimates for K_m and V_{max} were 37.09 mg/L and 353.57 mg/h respectively. The ME was -20.8 (95% CI -26.2; -15.4) mg/L while imprecision (RMSE) was 49.2 (95% CI 41.2; 56) mg/L

Conclusion: Piperacillin elimination is (partially) saturable. Moreover, the population estimate for K_m lies within the therapeutic window and therefore saturation of elimination should be accounted for when defining optimum dosing regimens for piperacillin in critically ill patients.

Keywords: piperacillin, pharmacokinetics, critically ill, saturation

Introduction

The ureïdopenicilin piperacillin combined with the beta-lactamase inhibitor tazobactam is frequently used to treat serious infections in critically ill patients [1,2]. In line with other beta-lactam antibiotics, piperacillin has time-dependent killing properties. The time (T) for which the free (*f*) concentration of piperacillin remains above the minimal inhibitory concentration (MIC) is the pharmacokinetic/pharmacodynamic (PK/PD) index of choice, i.e. $\%fT_{>MIC}$ [3].

In the past few years, a wealth of evidence emerged demonstrating that the PK of antimicrobial drugs in critically ill patients is profoundly different from the PK of antimicrobial drugs in healthy volunteers or non-critically ill patients [4]. For beta-lactam antibiotics specifically, changes in volume of distribution and/or changes in renal function in critically ill patients may lead to considerable between- and within-patient PK variability [5]. Previously, a pharmacokinetic point-prevalence study of beta-lactam antibiotics in the ICU reported that 16% of the ICU patients did not achieve the PK/PD target of $50\%fT_{>MIC}$ [6]. As suboptimal antimicrobial use may lead to poor infection outcome, efforts are made to optimize the use of beta-lactam antibiotics [7–9]. Because beta-lactam antibiotics have time-dependent killing properties, prolonging the duration of beta-lactam infusion and thereby extending the time the concentration remains above the MIC, was recently introduced in clinical practice [10,11].

Currently, there is an ongoing debate on whether or not piperacillin elimination is saturable at therapeutic plasma concentrations [12–19]. This mechanism is particularly relevant in the context of the recent introduction of prolonged infusion of beta-lactam antibiotics. Indeed, saturation of piperacillin elimination at therapeutic plasma concentrations implies that, for the total antibiotic exposure in a patient to be the same, a higher daily dose could be necessary when piperacillin is infused continuously as opposed to intermittently. In

clinical practice however, the total daily dose of piperacillin is usually not adapted based on the mode of infusion used [11,20].

The aim of this study was to investigate saturation of piperacillin elimination in critically ill patients receiving both intermittent and continuous infusion piperacillin.

Patients and methods

1. Patients

This prospective interventional study was conducted in the Department of Critical Care Medicine of Ghent University Hospital (Ghent, Belgium). Ethical approval was obtained from the Ghent University Hospital Ethics Committee (registration number 2017/1354). Informed consent was signed by patients or their representatives. Patients were eligible for inclusion if they were admitted to the surgical or medical ICU and received piperacillin/tazobactam (TZP) in continuous infusion. Patients younger than 18 years of age and patients receiving extracorporeal membrane oxygenation (ECMO) or renal replacement therapy (RRT) during antibiotic therapy were excluded from the study. Creatinine clearance was determined by measuring urinary creatinine concentrations from an 8-hour urinary collection using an indwelling urinary catheter. Piperacillin antibiotic concentrations and additional data such as, biochemistry, demographic data, the modified Sequential Organ Failure Assessment score (SOFA) on the day of sampling, the Acute Physiology and Chronic Health Evaluation (APACHE II) score on admission and ICU survival were prospectively recorded via REDCap [21].

2. Administration of piperacillin antibiotic therapy and sampling

All patients received both continuous and intermittent infusion TZP. TZP dosing was as follows: loading dose of 4/0.5 g /30 min immediately followed by a continuous TZP

infusion: (measured creatinine clearance (CL_{CR}) <15 mL/min: 8/1 g /24 h, CL_{CR} 15-29 mL/min: 12/1.5 g /24h and for a $CL_{CR} \geq 30$ mL/min 16/2 g/24h). At the end of the antibiotic course as indicated by the treating physician, after a 3-hour washout period, a short infusion (0.5 h; 4500 g) of TZP was administered. In total, 13 samples were collected from every patient. The first two samples were taken 2 hours prior to and immediately before stopping the continuous infusion. Samples 3-13 were collected immediately before administration of the intermittent infusion and after 5, 30, 45, 60, 90, 120, 180, 240, 300 and 360 minutes as shown in Figure 1.

3. Bioanalysis of piperacillin plasma concentrations

Arterial blood collected in 4 mL blood tubes (lithium heparin blood collection tubes, BD Vacutainer®, BD Diagnostics, Erembodegem, Belgium) was sent to the core laboratory of the Dept. of Laboratory Medicine at the Ghent University Hospital where they were first stored in a refrigerator at 4°C until they were collected by the toxicology laboratory technicians. Storage at 4°C was never longer than 24 hours. After transferring to an Eppendorf tube, plasma samples were centrifuged at 16162x*g* for 8 minutes (Microfuge 16, Beckman Coulter, Brea, California). Immediately afterwards, the plasma samples were stored at -20˚C until analysis. All samples were analyzed within 1 week. The plasma concentration of piperacillin was determined by ultra-performance liquid chromatography tandem mass spectrometry (UPLC – MS/MS). Tazobactam concentrations were not analyzed in this study. The lower limit of quantification (LLOQ) for piperacillin was 1.09 mg/L, the within-run assay imprecision at LLOQ level was 3.7 %CV and the between-run assay imprecision at the LLOQ level was 8.1 %CV [22].

4. Population pharmacokinetic model building

Piperacillin concentration-time data were analyzed using Pmetrics (version 1.5.2; Laboratory of Applied Pharmacokinetics, Los Angeles, CA, USA), an R-based software program for non-parametric and parametric pharmacokinetic-pharmacodynamic population and individual modelling and simulation. We used the non-parametric adaptive grid (NPAG) algorithm to build a PopPK model for piperacillin administered via continuous and intermittent infusion [23]. A digital Fortran compiler was used (Gfortran version 6.1; Free software foundation, Inc. Boston, MA, USA) and the runs were executed using R (version 3.5.1; The R Foundation for Statistical Computing. Vienna, Austria) and RStudio (version 1.1.383; RStudio, Inc. Boston, MA, USA). One- and two compartment models were fitted to the data using subroutines from the Pmetrics library. Modeling concentration-time data with both linear, parallel linear/Michaelis-Menten and Michaelis-Menten drug clearance was attempted. Subsequently, the statistical error model with the best fit was selected and a covariate model was developed. Covariates *a priori* considered for inclusion in the model were: measured creatinine clearance, estimated creatinine clearance (Cockroft-Gault formula), estimated glomerular filtration rate (Modification of Diet in Renal Disease (MDRD) formula), body weight, age, SOFA score and albumin, based on prior knowledge and biological plausibility [4,24–27]. Body weight was included as a primary covariate on all model parameters, except for K_m and V_{max}, according to the allometric power model [28].

(1) $P\ \theta_i = TVP\theta_1*(WEIGHT/70)**power$ Eq. 1

Where $P\ \theta_i$ is the individual parameter value, $TVP\theta_1$ is the parameter value for a typical adult with a body weight of 70kg, and power is an allometric exponent fixed to 0.75 for CL and Q and fixed to 1 for V and Vp. As an initial step, covariates measured creatinine, estimated creatinine clearance via Cockroft-Gault formula and estimated glomerular filtration rate using the MDRD formula were tested on the CL parameter as this is biologically plausible.

However, only one of these was retained as correlated variables may lead to collinearity and inflation of the parameter's standard error [29]. In a next step, forward selection and backward elimination using the PMstep function in Pmetrics was used to assess the relationship between covariates and model parameters. The log likelihood ratio test (LRT) and the Akaike information criterion (AIC) were considered during model building. More specifically, a difference of 3.84 in the log likelihood was considered significant at the 5% level when performing the likelihood ratio test for comparing nested models. Estimated parameters are reported as mean, percent coefficient of variation (%CV) and median with interquartile range (IQR). The %CV is reported as a measure of between-subject variability in the model parameters. 95% Confidence intervals were estimated *via* a non-parametric bootstrap (n=1000) and quantify the uncertainty on the parameter estimates.

5. Pharmacokinetic model diagnostics

The PopPK model was assessed by visual evaluation of the goodness of fit of the observed versus *a posteriori* predicted plots and the coefficient of determination of the linear regression of the observed-predicted values (r^2 close to 1, intercept close to 0) from each run. The predictive performance was assessed on mean prediction error (bias) and the mean bias-adjusted square prediction error (imprecision) of the population predictions.

Internal model validation consisted of a visual predictive check (VPC) plot. The VPC (n=10.000) was performed by overlaying the 95% CI of the simulated profiles for 0.05, 0.5 and 0.95 quantiles with the corresponding quantiles of the observed data.

For external model validation, the final model population parameter distributions were used to predict concentrations for an independent validation dataset. We refer to Dhaese, *et al* [30] for a detailed description of this validation dataset. Prediction errors were evaluated based on the absolute bias (ME) and imprecision (MSE) as described in equation 2 and 3:

(2) Absolute bias[$\hat{\theta}$] (ME) = E[$\hat{\theta} - \theta$] Eq.2

(3) Absolute imprecision[$\hat{\theta}$] (MSE) = E[$(\hat{\theta} - \theta)^2$] Eq.3

Where $\hat{\theta}$ is the predicted piperacillin concentration and θ is the observed concentration. The root mean square prediction error (RMSE) was calculated by taking the square root of MSE.

6. Comparative AUC_u simulations for intermittent and continuous infusion dosing regimens

Monte Carlo simulations (n=1000) were performed with the final PopPK model to compare the unbound (u) area under the curve (AUC_u) as a measure of total (unbound) drug exposure between intermittent and continuous infusion dosing regimens. Using AUC as a basis to compare intermittent and continuous infusion of beta-lactam antibiotics was previously reported by Firsov and Mattie [31]. Free piperacillin concentrations were calculated assuming a 30% level of protein binding in accordance with previous findings [32]. Four different scenarios were evaluated; i.e. a daily dose of 12/1.5g TZP for a patient with a measured CL_{CR} of 20mL/min, 16/2g TZP for a patient with a measured CL_{CR} of 70mL/min, 16/2g TZP for a patient with a measured CL_{CR} of 130mL/min and 16/2g TZP for a patient with a measured CL_{CR} of 200mL/min. The body weight for all patients was fixed at 70kg. For each of these four scenarios, both intermittent and continuous infusion dosing regimens were simulated and compared. The AUC_u was calculated using linear trapezoidal approximation. A 24-hour interval for AUC_u calculation was chosen after six doses for intermittent infusion and one bolus and five maintenance doses for continuous infusion.

7. *Post hoc* estimation of type I error rate

A type I error rate analysis was performed to evaluate the probability to reject the null-hypothesis (H_0) in favor of the alternative hypothesis (H_1) given that it is true, where H_0 =

piperacillin kinetics are best described by linear elimination and H_1= piperacillin kinetics are best described by non-linear elimination. [27]

In short, we simulated concentrations for 17 patients according to the design of this study (drug administration, blood sampling, etc.). For this, the PopPK model by Landersdorfer, *et al* [12] served as the H_1, i.e. piperacillin PKs are non-linear and elimination is characterized by a parallel first-order and Michaelis-Menten process. The H_0 was simulated by fixing the V_{max} estimate in the model by Landersdorfer to zero, i.e. removing the non-linear component in piperacillin elimination. This process was repeated 5000 times, resulting in 10,000 simulated datasets. All simulated datasets were fitted with a two-compartmental model with linear elimination and a two-compartmental model with parallel linear and Michaelis-Menten elimination. Both models were compared using the LRT according to equation 4.

(4) $LRT = 2*(LL_c - LL_r)$ Eq. 4

where LL_c is the log likelihood (LL) for the more complex model and LL_r is the LL for the reduced model. The difference in the number of parameters between both models was 4 when between-subject variability was included in the estimation of K_m and V_{max} and was 2 otherwise. When considering the 5% level of significance, the critical values from the chi-square distribution were 9.49 and 5.99, respectively.

The type I error rate was calculated from the number of times the complex model was declared superior over the reduced model for the simulated datasets according to the H_0.

8. Statistical analysis

All statistical analyses were performed using R and RStudio. Continuous data are presented as median (interquartile range). Categorical data are presented as counts (%).

Results

1. Patients and samples

In total, 17 patients were included, and 221 samples were collected (Table 1). All patients were enrolled between 5/2/2018 and 18/10/2018. Samples 5-7 were lost for patient 13 and sample 8 was lost for patient 15, therefore only 217 samples were analyzed and used for PK model building. The focus of infection was respiratory in 11 patients, abdominal in 5 patients and bacteremia in 1 patient.

2. Pharmacokinetic model building and model diagnostics

Table 2 summarizes the log-likelihood values, the coefficients of determination (r^2 values), the AIC's and the predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten models (without covariates). Comparison of the coefficient of determination, the bias, imprecision and AIC indicated that the model with parallel linear and Michaelis-Menten kinetics was superior compared to both a model with linear elimination and a model with Michaelis-Menten elimination alone (Table 2).

Including measured creatinine clearance (mCRCL) normalized to 100 mL/min as opposed to estimated creatinine clearance using the Cockroft-Gault or the estimated glomerular filtration rate using the MDRD formula provided the model with the lowest AIC value (Table 3). Forward selection and backward elimination further revealed a relationship between albumin and clearance. However, when including albumin as a covariate on CL, no model improvement in terms of ΔAIC or LRT was noted, hence albumin was not retained as a covariate in the final model.

The final model was described as:

(5) $CL = TVCL*(mCL_{CR}/100) *(WEIGHT/70)**0.75$ Eq. 5

(6) $V = TVV*(WEIGHT/70)$ Eq. 6

(7) $Vp = TVVp * (WEIGHT/70)$ Eq. 7

(8) $Q = TVQ^* (WEIGHT/70)^{**}0.75$ Eq. 8

where CL is piperacillin clearance, V is volume of distribution of the central compartment, Vp is volume of distribution of the peripheral compartment and Q is the intercompartmental clearance. TVCL refers to the population typical piperacillin clearance for a 70-kg patient with a mCL_{CR} of 100 mL/min, TVV and TVVp refer to the population typical volume of distribution of the central, respectively the peripheral compartment for a 70-kg patient.

The mean, %CV, median (IQR) and %95 CI around the median for the population parameter estimates are listed in Table 4. The typical value for K_m and V_{max} was 37.09 mg/L and 353.57 mg/h respectively.

Between-subject variability was not estimated on K_m and V_{max} as this resulted in an over-parameterized model and an unacceptable inflation of the type I error rate (for further details see the section "*Post hoc* estimation of type I error rate"). Based on the diagnostic plots, the γ multiplicative error model was selected for modelling assay variance. In all model-building runs, each observation was weighted by $1/ (\gamma \times SD^2)$. We set γ equal to 1 initially and allowed Pmetrics to fit the value for the population. The final-cycle γ value was 1.26, indicating some additional process noise. The formula for the γ error model is error= γ*SD where SD is the standard deviation of each observation. SD is modeled by equation 9 and was based on earlier validation work by Carlier, *et al* [33].

(9) $SD = 2 + 0.1x\ C$ Eq. 9

where C is the concentration of piperacillin.

The *a posteriori* individual and population predicted versus observed plots and the VPC plots are shown in figure 2 and 3 respectively. The results of the Shapiro-Wilk test of normality for the NPDE indicated no violation of normality (p=.195).

The final PopPK models showed a bias (ME) in predicting serum concentrations from the validation dataset of -20.8 (95% CI -26.2 ; -15.4) mg/L while imprecision (RMSE) was 49.2 (95% CI 41.2 ; 56) mg/L. The Bland-Altman plot is shown in figure 4.

3. Comparative AUC_u simulations for intermittent and continuous infusion dosing regimens

In all four scenarios, patients receiving continuous infusion had lower AUC_u values when compared to simulated patients receiving the same dose *via* intermittent infusion (figure 5).

4. *Post hoc* estimation of type I error rate

If the between-subject variability was estimated for all model parameters, the type I error rate was 47.9%. If the between-subject variability was estimated for CL, Q, V and Vp and not estimated for K_m and V_{max}, the type I error rate was reduced to 6.6%.

Discussion

A PopPK model with parallel linear and Michaelis-Menten elimination of piperacillin best described this data, collected from 17 critically ill patients receiving both intermittent and continuous infusion piperacillin/tazobactam. These findings are in agreement with previous studies in healthy volunteers and non-critically ill patients [12,13,17] and in disagreement with other studies in healthy volunteers and critically ill patients [14,30,34].

Renal excretion of piperacillin is the major pathway of elimination. Approximately 74-89% of the administered dose of piperacillin is eliminated from the body by renal excretion [2,35]. More specifically, Tjandramaga, *et al*. [35] reported that 56-73% of the renally cleared piperacillin is eliminated through tubular secretion, which is a saturable process.

V_{max} is the maximum elimination rate for Michaelis-Menten elimination and the drug concentration at which the elimination rate is half of the maximum elimination rate is called the Michaelis-Menten constant or K_m. Whether or not non-linear elimination of a drug is clinically relevant depends on the value of V_{max} and K_m. Non-linear elimination is a clinically relevant process if saturation occurs at therapeutic concentrations (i.e. K_m within the therapeutic window) and if V_{max} is high relative to CL, indicating a substantial contribution of the non-linear elimination process to the total body clearance. It is postulated that the non-linear elimination pathway should contribute to at least 20% of the total body clearance for it to be clinically relevant [36]. If K_m is very high, then saturation occurs but not at relevant plasma concentrations and it will therefore have no impact on the optimal dosing regimen [12]. Other researchers have reported K_m estimates of 36.1 mg/L [12], 47.9 mg/L [13] and 90.13 mg/L [17], all well in the range of therapeutic piperacillin plasma concentrations and in line with our estimate of 37.09 mg/L.

The implications of these findings remain to be determined. Several institutions recently moved towards prolonged infusion of beta-lactam antibiotics yet conclusive evidence in favor of prolonged infusion is lacking and new clinical trials are in the pipeline [10,11,20,37]. Saturation of piperacillin elimination at therapeutic plasma concentrations is of particular relevance when randomized clinical trials compare intermittent versus continuous infusion piperacillin. Indeed, if saturation of piperacillin elimination occurs at therapeutic concentrations, clinical trials comparing the same daily dose of intermittent and continuous infusion piperacillin may unwillingly introduce a bias towards intermittent infusion as patients receiving the same daily dose of piperacillin *via* intermittent infusion may have a higher total antibiotic exposure when compared to patients receiving the same dose of piperacillin *via* continuous infusion as is demonstrated in the $AUC_{u\ 24}$ calculations using the final PopPK model (figure 5). While AUC_u/MIC may not be the PD index of choice for beta-

lactam antibiotics, the phenomenon of non-linear kinetics may impact antibiotic concentrations and indirectly also other PD indices such as $T_{>MIC}$. This study focused on piperacillin but tubular secretion of other beta-lactam antibiotics such as amoxicillin, oxacillin, flucoxacillin, cefazolin and cefuroxime has been reported as well [38,39].

When performing hypothesis testing and PK model selection, control of the type I error rate is pivotal to avoid false positive conclusions. Inflation of the type I error rate is expected when dealing with (very) small datasets [40,41]. In this study, including the between-subject variability on K_m and V_{max} resulted in an over-parameterized model and an unacceptable type I error rate (for further details see the section "*Post hoc* estimation of the type I error rate"). Therefore, the between-subject variability for K_m and V_{max} was not estimated. As few piperacillin population PK studies incorporate type I error calculations, it is difficult to determine how our findings with regard to the non-linear kinetics of piperacillin relate to the findings of other studies.

This study has several limitations. While our primary goal was to detect non-linear elimination of piperacillin with a low probability of falsely rejecting H_0, the between-subject variability was not estimated on K_m and V_{max} as this led to an unacceptable type I error. Determining urinary concentrations of renally eliminated drugs is helpful when non-linear kinetics are expected, however, in this study, piperacillin concentrations were not measured in the urine and no distinction could be made between the renal and non-renal clearance of piperacillin. The validation results indicate that the final model has a bias towards underpredicting antibiotic concentrations. While no bias is to be preferred, in case of underprediction, physicians may be inclined to increase the dose or dosing frequency. Given the low toxicity of beta-lactam antibiotics and the important risk of underdosing in ICU patients, models that underpredict concentrations of beta-lactam antibiotics are usually preferred over models that have bias towards overprediction [42]. Additionally, the sequence

of the infusion modes never changed and all patients received continuous infusion first, followed by intermittent infusion. Hence, a trend in piperacillin clearance over time could not be excluded.

In conclusion, piperacillin elimination was best described by a PopPK model incorporating parallel linear and Michaelis-Menten elimination. Nevertheless, in literature conflicting evidence is found on the importance of non-linear elimination for piperacillin PK. Non-informative study designs, and statistical inference based on over-parameterized models likely contribute to these conflicting findings. Future studies, appropriately powered and with a low type I error rate, should be conducted to provide conclusive evidence on the potential influence of non-linear elimination for piperacillin PK in critically ill patients.

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Jan De Waele has been consultant for Accelerate Diagnostics, Bayer Healthcare, MSD and Pfizer

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Captions and legends of tables and figures

Tables

Table 1: Patient characteristics, laboratory data and infection characteristics

Table 2: Predictive performance of linear and non-linear piperacillin population PK models

Predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten model. R^2 is the coefficient of determination for the best-fit linear regression for the predicted-observed plot. LL is the log likelihood estimate. AIC is the Akaike information criterion. L = linear, MM= Michaelis-Menten.

Table 3: Predictive performance of piperacillin population PK models incorporating renal clearance as a covariate

Predictive performance of linear, parallel linear and Michaelis-Menten and Michaelis-Menten model. R^2 is the coefficient of determination for the best-fit linear regression for the predicted-observed plot. LL is the log likelihood estimate. AIC is the Akaike information criterion. mCL_{CR} = measured creatinine clearance, GaG = estimated creatinine clearance using the Cockroft-Gault formula, MDRD = estimated glomerular filtration rate using the MDRD formula.

Table 4: Mean, %CV, median (IQR), and 95%CI parameter estimates for the final PopPK model

Figures

Figure 1: Administration of piperacillin and timing of sampling

Figure 2: The population predicted versus observed concentrations (left) and the individual predicted versus observed concentrations (right) diagnostic plots for the final PK model. The dashed line is the line of unity and the solid line is the line of the best linear fit.

Figure 3: Visual predictive check plot of piperacillin plasma concentrations (log10 scale) vs. time for the final PopPK model. Black dots represent observed data, solid lines represent quantiles of the observed data and dashed lines represent quantiles of the simulated data.

Figure 4: Bland-Altman plot for comparison of predicted versus observed piperacillin concentrations from a validation dataset. The blue line represents the mean difference in concentrations. Red lines are mean-1.96*SD (lower line) and mean+1.96*SD (upper line).

Figure 5: Simulations of mean (sd) AUC_u values and time-concentration curves for a total daily dose of 12/1g PIP (upper graph) or 16g PIP via intermittent (left) or continuous (right) infusion for a patient with a body weight of 70kg and a measured CL_{CR} of respectively 20, 70, 130 and 200mL/min. AUC_u values were calculated for a 24-hour interval after the sixth dose.